IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of: **Gilmore**	)	
	)	Group Art Unit: 1645
Serial No.: 09/004,395	)	
	)	Examiner: N. Minnifield
Filed: December 23, 1996	)	
	)	Docket No. 97,429

For: **RECOMBINANT P37/FLAA AS A DIAGNOSTIC REAGENT**

BRIEF ON APPEAL

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Three copies of this appeal brief are submitted along with the large entity fee of three hundred ten dollars ($ 310.00) for filing an appeal. A notice of appeal was filed on November 22, 2000. A petition for a two-month extension of time was filed on January 29, 2001, along with a fee of three hundred ninety dollars ($390). Appellants respectfully petition for an additional three-month extension of time, for a total of five months of extension. A fee of one-thousand dollars ($1,500) is enclosed.

In the event of any variance between any of the amounts enclosed and the Patent

Contents

I. REAL PARTY IN INTEREST

The inventors of the claimed invention have assigned the rights to this application to bioMerieux, Inc., St. Louis, Missouri. The assignee is a wholly owned subsidiary of a French company, bioMerieux, SA, a privately held company.

II. RELATED APPEALS AND INTERFERENCES

No pending appeals, interferences or applications directly affects or has a bearing on a decision in this appeal.

II. STATUS OF CLAIMS

Claims 14-17 and 19-30 are pending and are provided in Exhibit A.

Claims 14-17 and 19-30 stand rejected under 35 USC §112, second paragraph.

Claims 14-17 and 19-30 also stand rejected under 35 USC §102(a).

III STATUS OF AMENDMENTS

An Office Action finally rejecting Claims 14-17 and 19-30 was issued on May 23, 2000. A response to the Final Office Action was filed on November 22, 2000. The response canceled Claims 19, 27 and 30 and proposed amendments to Claims 14-17, 20-26 and 28-29.

An Advisory Action dated December 14, 2000 indicated that the amendments to the claims made after final would not be entered because they required further

better form for appeal. The amendments are necessary to respond to the Examiner's 35 U.S.C. §112, second paragraph rejections, which were made for the first time in the Final Office Action. The amendments were not earlier presented because the Appellants believed that the claims were in form for allowance. Appellants respectfully request entry of the Supplemental Amendment.

A copy of appealed claims 14-17 and 19-30 are found in Appendix A.

IV SUMMARY OF THE INVENTION

Applicants have discovered that the recombinant FlaA or P37 protein is an important antigen for detection of Lyme disease. FlaA and P37 are now recognized in the art as being the same protein. Applicants' invention also provides a diagnostic test for early detection of Lyme disease utilizing a recombinant FlaA protein. The invention further encompasses manual or automated assays to detect antibodies to Lyme disease by direct detection of a FlaA or P37 protein immobilized on a solid support or in solution.

The invention is also drawn to methods for the production of a recombinant FlaA or P37 protein from transformed cell cultures *B. burgdorferi*. The invention further encompases the production of recombinant FlaA protein for uses other than in a test kit. The recombinant FlaA or P37 protein can be obtained by constructing a DNA expression vector, transforming a host cell with the expression vector, preparing cell cultures from **fresh transformants** from the host cell, inducing FlaA or P37 protein expression and isolating the recombinant FlaA or P37 protein therefrom (emphasis added). The FlaA or

Appealed claims 14-17 and 19-30 are provided in Exhibit A. Claims 14-19, 27-30 are product claims directed to a diagnostic reagent. Claims 20-25 are product-by-process claims directed to a diagnostic reagent made by a method for producing recombinant FlaA or P37 protein. Support for the claims pending in this appeal can be found in the specification as a whole and more specifically, for example, at pages 12-18.

V ISSUES

The issues on appeal are:

(a) Whether Claims 14-17 and 19-30 are unpatentable under 35 USC §112, second paragraph rejection.

(b) Whether Claims 14-17 and 19-30 are unpatentable under 35 U.S.C. §102(a) as anticipated by Ge *et al.*, *J. Bacteriol.* 179(2):552-556 (1997)(**Ge I**);

(c) Whether Claims 14-17 and 19-30 are unpatentable under 35 U.S.C. §102(a) as anticipated by Ge *et al.*, *Infect. Immun.* 65(7):2992-2955 (1997) (**Ge II**); and

(d) Whether Claims 14, 20 and 24 are unpatentable under 35 U.S.C. §102(a) as anticipated by Fikrig et al., WO 97/42325.

VI GROUPING OF CLAIMS

The claims do not all rise or fall together. The claims are separately argued in accordance with the following grouping of claims:

Group I. Claims 14-19, 27-29 and 30 of Exhibit A are considered one group, and rise or fall with Claim 14. If entered, amended claims 14-17, 28 and 29 as considered one group, and rise or fall with Claim 14

Group II Claims 20-26 in Exhibit A are considered one group and rise or fall with Claim 20. If entered, amended claims 20-26 as presented in the supplemental amendment (Exhibit B) are considered one group and rise or fall with Claim 20.

VII ARGUMENT

A. The appealed claims do not rise or fall together

The claims of Group I are separately patentable from the claims of Group II. Group I includes product claims directed to a diagnostic reagent used in a test kit. Group II claims are directed to a product made by a process. The claims are separately patentable because the product of Group II is produced by a combination of specific processes including preparation of host cells from a fresh transformant colony and may have uses other than as a diagnostic reagent.

B. FlaA and P37 are the same protein in the invention

The FlaA protein or P37 are considered by Applicants to be the same material. As noted in Applicants' Paper No. 17 the ambiguity regarding the use of P37 to describe two different proteins in *B. burgdorferi* has led to confusion in the scientific literature. The term "P37" had been used generally in the art to describe proteins having a 37 kDa molecular weight. Two 37 kDa proteins have been identified from the *B. burgdorferi* tick; a P37 protein isolated by Fikrig, et al. (Immunity, 6:531-529 (1997)) and a second P37 protein or the FlaA protein of the present invention. Recently, the confusion in nomenclature has been resolved in an article by Feng, et al. (*Infection and Immunity*

"understanding in the use of nomenclature and has also distinguished the P37 protein

(See Feng at page 4172, column 1, first paragraph of the Discussion) The P37 protein of Fikrig is genetically different from the FlaA protein in the invention.

Applicants' reference to the "P37" protein in the appealed claims is in fact the same as the FlaA protein as claimed and supported throughout the specification. In order to minimize any further confusion and to adopt the new nomenclature Applicants would agree upon entry of the amendments to delete the term "P37" from the appealed claims as provided in Exhibit B. (*Schering Corp. v. Amgen Inc.*, 55 USPQ2d 1650, 1654 (CAFC 2000)(substitute terminology was not a new matter violation in view of patent's written description).

C. Group I

Group I consists of claims 14-19, 27-29 and 30 (Exhibit A), which rise or fall with claim 14. Claim 14 of Exhibit A recites the following subject matter:

> 14. A diagnostic reagent for early detection of Lyme disease comprising a recombinant FlaA or P37 protein.

1. Claims 14-17 and 19-30 are definite under 35 USC §112, second paragraph because one of skill in the art would understand what is claimed when read in light of the specification

Claims 14-17 and 19-30 stand rejected as being indefinite under 35 U.S.C. §112, second paragraph. The Examiner alleges the claims are vague and indefinite in the recitation of "recombinant FlaA or P37 protein" because it is allegedly unclear whether "FlaA or P37" refers to the same protein.

The meaning of "FlaA or P37" has now been resolved in the scientific literature.

The FlaA protein of the present invention is in fact the same as the P37 protein claimed and differs only in nomenclature. As noted above, at the time of the invention, the art was accustomed to using these terms interchangeably as Applicants have provided in their specification.

In a recent clarifying publication, Feng, et al. *Infection and Immun*, Vol. 68, No. 7, p. 4169-4173, 4172 (July 2000) has rectified and corrected the nomenclature of the 37 kDa protein from *B. burgdorferi* and has distinguished the P37 protein from FlaA proteins. Specifically,

> We report here two additional immunoreactive 37-kDa lipoproteins, one of which we have designated Arp. These findings reinforce the need to name genes and gene products based upon function rather than molecular weight to avoid confusion (Feng, *Infection and Immun.*, p. 4172)

The Examiner has keenly recognized a potential for confusion. The ambiguity in nomenclature arose in the early technical literature where the FlaA protein of *B. burgdorferi* was generally named by its molecular weight of 37 kDa without further distinction. The P37 protein isolated by Fikrig, WO 97/42325 and the FlaA or P37 protein of the present invention are not now accepted as being the same protein.

Applicants' use of the terms FlaA and P37 are consistent with that of Feng et al. Since the FlaA and P37 protein described in the specification at page 13, lines 14-15 are properly identified as being the same, no ambiguity exists in the recitation of "FlaA or P37" in the claims. One of skill in the art reading the specification would understand the language of the claims and the scope of the intended invention as Applicants have

clear in the specification and the meaning is consistently adhered to in determining patentability and validity. (See, *Markman v. Westview, Instruments, Inc.* 34 USPQ2d 1321, 1330 (Fed Cir. 1995)(en banc) *aff'd*, 38 USPQ2d 1461 (1996)).

Alternatively, Applicants would agree upon acceptance of the claim amendments appearing in the attached supplemental amendment (Exhibit B) that accompanies this Appeal Brief in hope of resolving this Section 112 issue. In the attached Exhibit B, claims 14, 16, 20 and 24 have been amended to delete "P37".

Additionally, the following pending claims on appeal (provided in Exhibit A) stand rejected as being vague and indefinite under 35 U.S.C. §112, second paragraph:

(a) claims 15-17, 22 and 23 in the recitation of "partial amino acid sequence";

(b) claim 19 in the recitation of "said protein having the amino acid sequence of amino acids 1-39 of SEQ ID NO:2" and because it is allegedly unclear whether the same sequence is in both FlaA and P37 proteins; and

(c) claim 30 in the recitation of "substantially" and "substantially antigenic" because it is allegedly unclear how much of the amino acid sequence is necessary to determine "substantially".

The Examiner's rejection of these terms is respectfully traversed because one of skill in the art would recognize what the Applicants have claimed as their invention. The claim language rejected above is clear, concise, well-known as used in the art and is described in various scientific instruction manuals as well as in the specification. See for example Sambrook, et al., Molecular Cloning, 2d edition, Cold Spring Harbor Press

Sobel, 1992; Rose, et al., Manual of Clinical Laboratory Immunology, 3d edition.

American Society of Microbiologists (1986); and see the specification at pages 9-11 (Example 1 Isolation and identification of a P37 gene clone). These manuals and references are examples of typical texts readily available to the skilled artisan and provide conventional teaching of methodologies used in the art.

Applicants' specification discloses various methodologies and routine experiments as known to persons of ordinary skill in the art to determine for example, partial amino acid sequences, substantially antigenic regions of the amino acid sequences, etc. to achieve the use of FlaA as a diagnostic reagent (specification, pages 8-18). Specifically, the entire amino acid and the entire nucleic acid sequence of the FlaA protein and gene are taught in the specification as well as in Ge, I or II. It would be routine experimentation and within the skill of the artisan to express and isolate partial amino acid sequences to produce substantially antigenic fragments from nucleic acid sequences and their complements. One of skill in the art is clearly apprised of the metes and bounds of the specification given the teaching therein.

The test for definiteness of a claim under 35 USC §112, second paragraph is whether the claim meets the threshold requirements of clarity and precision, whether the claim language is precise and defines the patentable subject matter with a reasonable degree of particularity and distinctness. "The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. In cases involving predictable factors...a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be

known scientific laws." See *In re Fisher*, 166 USPQ 18, 24 (CCPA 1970). Applicants

submit that the scope and language of the claims are definite and properly define Applicants' invention. The rejection of claims 15-17, 19, 22, 23 and 30 under 35 USC §112 should be withdrawn.

While Applicants believe that the claim language is clear, entry of amendments to claims 15, 22, 23 and cancellation of claims 19 and 30 as provided in the attached supplemental amendment (Exhibit B) have been provided in the alternative.

Claims 27-29 stand rejected under 35 USC §112, second paragraph as being vague and indefinite because there is allegedly insufficient antecedent basis for the limitations in the claim. Applicants acknowledge the error is in reciting the wrong claim dependencies, for example, the amino acid sequences in claim 27 depends incorrectly from the nucleic acid sequence in claim 14. Applicants respectfully request cancellation of claims 27 and 30 and entry of amendments to claims 28 and 29 as provided in the attached supplemental amendment (Exhibit B) to correct dependencies. It is unclear why the Examiner did not accept these proposed amendments to correct the rejection for antecedent basis.

Claims 14-17 and 19-30 are definite such that one of skill in the art would understand what is claimed when the claim is read in light of the specification. The rejection of claims 14-17 and 19-30 under section 112 should be withdrawn.

2. The claims are not anticipated by either Ge I or Ge II because neither reference teaches each and every element of the claim under 35 USC §102(a)

product by process) are directed to a recombinant FlaA protein or P37 protein. In the Advisory Action the Examiner further alleged that the language "diagnostic reagent" for the detection of Lyme disease" in the claims is viewed as an "intended use that has no standing with regard to the anticipation rejections." The Examiner's interpretation of the claims are incorrect. The art cited by the Examiner does not establish a proper *prima facie* rejection under Section 102(a). Each of the references fail to disclose each and every element as provided in the claims on appeal.

Neither Ge I nor Ge II or even the combination of these references anticipate the invention because they do not individually teach a diagnostic reagent as claimed in the present invention. Ge I does not disclose, expressly or impliedly, the utility of FlaA protein as a diagnostic reagent. Ge II expressly advises against the use of the FlaA protein in diagnosing Lyme disease. Ge II concluded:

> FlaA **is not an immunodominant antigen** in Lyme disease. (second column, heading, p. 2993)(emphasis added)

and

> ...FlaA is a protein unique to spirochetes, our results suggest that it **is not a good candidate** for the serodiagnosis of Lyme disease. (second column, last sentence, p. 2994)(emphasis added).

Ge II could not more clearly express their mistaken belief that FlaA is a suitable antigen to pursue in a test kit or diagnostic test for Lyme disease than in the title of the article: "**FlaA, a Putative Flagellar Outer Sheath Protein, Is Not an Immunodominant Antigen Associated with Lyme Disease.**"

P37" to detect early Lyme disease. That FlaA or P37 is suitable for use in a diagnostic test is exactly what Ge II found unworkable.

In order to find anticipation or lack of novelty under 35 USC §102(a), every limitation of a claimed invention must be taught, either explicitly or inherently, within a single prior art reference. *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989), *Glaxo Inc. v. Novopharm Ltd.*, 34 USPQ2d 1565, 1567 (Fed. Cir. 1995). The Examiner has not met a *prima facie* burden to show each and every element of the claims on appeal in Ge I or Ge II. This rejection should be withdrawn.

The Board's attention is further directed to the preamble of claim 14, "A diagnostic reagent for early detection of Lyme disease...." The preamble of the present claims is not merely a statement of purpose or use but also gives the claim meaning and scope. Applicants' preamble is significant because it defines their invention. *Kropa v. Robie and Mahlman*, 88 USPQ 478, 481 (CCPA 1951) (a preamble is given the effect of a limitation where the introductory words "give life and meaning" to the subject matter defined by the claims). Anticipation was not found where without the essential meaning provided in the preamble of the claim, the structures of the claim alone did not define the invention and the problems solved by the inventors. See *Corning Glass Works v. Sumitomo Electric U.S.A. Inc.*, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989). In Corning, the issue was whether the preamble ("an optical waveguide") was a limitation in the claim. Sumitomo argued that the structure recited in the claim at issue was identical to a previously disclosed conventional fiber structure

[illegible] had no effect or limitation, then the claims would be anticipated by

the previously disclosed conventional fiber structure, otherwise they were not. *Id.* Like the optical waveguide in the Corning case, the preamble in the instant case is the subject matter being worked on to solve the problem of providing an effective test for diagnosing early Lyme disease. A claim preamble should be given importance as part of the claim if the preamble, in conjunction with the body of the claim defines "one unified and internally consistent recitation of the claimed invention." *Pitney Bowes, Inc. v. Hewlett Packard Co.*, 51 USPQ2d 1162, 1166 (Fed. Cir. 1999) Claims 14-17 and 19-30 are defined by both the preamble and the claim body together directed to a diagnostic reagent utilizing a recombinant FlaA or P37 protein, and not solely a recombinant protein.

Whether preamble recitations are considered additional structural limitations, statements of use or mere introductory language is determined by examining the entire record for the intended invention sought to be claimed. *Id.* at 1966. As further support of the intended invention, the entire specification sets forth detail specifically teaching a recombinant FlaA or P37 protein as an effective reagent in a test kit. Here, both the specification and claims define the intended invention, a diagnostic reagent including FlaA or P37.

Thus, to read the claims and specification separately as the Examiner has done, is to dismiss the subject matter of the specification and then to substitute incorrect subject matter for what is being claimed is improper.

Consequently, withdrawal of the 35 U.S.C. §102(a) rejection of claims 14-17 and 19-30 of Exhibit A is in order and respectfully requested. In the alternative, claims

rejected are also not anticipated under section 102(a) for the reasons described above.

3. An Anticipating Reference Must Describe The Claimed Invention Sufficiently To Have Placed One Of Skill In The Art In Possession Of The Subject Matter of the Claims

The second step in an anticipation analysis is a comparison of the claims to the prior art references. In addition to a requirement that each and every limitation of the claimed invention be found, the reference must also be enabling and describe the claimed invention "sufficiently to have placed it in possession of a person of ordinary skill in the field of the invention." *In re Paulsen*, 31 USPQ2d 1671, 1673 (Fed. Cir. 1994). Since Ge I or Ge II do not teach one of skill in the art how to accomplish a diagnostic assay with FlaA as a reagent or how to analyze results and data of such assay, neither of the references anticipate the claims presented. The Examiner has misconstrued what the reference and data actually teaches and disregarded what is claimed in the present invention.

The Ge I or Ge II references teach that the FlaA protein "does not appear to be a consistent immunodominant antigen in infected mammalian hosts." See *Infect. Immun.* p. 2994. Ge II presents the following table at page 2994:

TABLE 3. Serological analysis of FlaB and FlaA

SERUM

Protein	Mouse (tick bite infected)	Rabbit (tick bite infected)	Rabbit (intravenously infected)	Monkey (early)[a]	Monkey (late)[b]	Human (19)[c]
FlaB[d]	+	+	+	+	+	+ (19)
FlaA[d]	-	-	-	-	-	+ (2)
FlaAR[e]	-	-	-	-	-	+ (2)

[a] Early, 14 weeks postinfection.
[b] Late, 164 weeks postinfection.
[c] Numbers in parentheses are the number of serum samples tested.
[d] Native protein.
[e] Recombinant protein.

The data shows that only 2 out of 19 patients were reactive with recombinant FlaA, a mere 10% reactivity. A result of 10% is not a dispositive showing that Ge II had possession directly or inherently of a diagnostic reagent for Lyme disease. One of skill in the art following the data of Ge I and Ge II would not be led to consider the use FlaA as a diagnostic reagent in a test kit.

In contrast, Applicants teach a diagnostic reagent that is an FlaA protein for use in diagnosing Lyme disease. The entire specification sets forth detail specifically limiting the use of FlaA as an effective diagnostic reagent for detecting early Lyme disease. The invention as a whole as described in the specification further teaches a recombinant FlaA protein as a diagnostic reagent. For example, in the specification at page 14, lines 5-15, presents data showing 100% reactivity of serum samples against the recombinant FlaA protein of the invention. This data is a significant teaching that Applicants have

on establishing using FlaA protein as a diagnostic agent. Furthermore, the claims are limited to a diagnostic reagent not exclusively the FlaA or P37 protein or its sequence.

The Examiner also improperly concludes that the subject matter of the claim, a diagnostic reagent, is "a product, the protein, which the prior art sets forth" (Final Office Action, page 4, last paragraph) because of its identity with the FlaA protein isolated and characterized by Ge I. The Examiner's rejection seems to assume that identity of the protein inherently produces the diagnostic reagent as claimed. At best the teachings of Ge I or II are a general invitation to use FlaA in detecting Lyme disease.

The CCPA has stated:

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. If, however, the disclosure is sufficient to show that the natural result flowing from the operation as taught would result in the performance of the questioned function, it seems to be well settled that the disclosure should be regarded as sufficient. *In re Oelrich and Divigard*, 212 USPQ 323, 326 (CCPA 1981).

Nowhere in Ge I or II is there teaching of FlaA as a diagnostic reagent.

The Examiner's rejection is therefore improper. Accordingly, withdrawal of the 35 U.S.C. 102(a) rejection of claims 14-17 and 19-30 of Exhibit A is in order and respectfully requested. Alternatively, for the reasons discussed above, the claims presented in the attached supplemental amendment (Exhibit B), if entered, are also not anticipated by the references.

4. Claims 14, 20 and 24 are not anticipated by Fikrig because Fikrig does not teach each and every element of the claim under 35 USC §102

interpretation of the Fikrig disclosure is incorrect. The P37 protein of Fikrig is not the same as the 37 kDa FlaA or P37 protein of the present invention. The P37 nucleic acid sequence of Fikrig (SEQ ID NO: 6) does not have the same or even complementary nucleic acid sequence as the FlaA sequence (SEQ ID NO: 2) in the present invention.

As discussed above, two 37 kDa proteins were originally identified in *B. burgdorferi*. The 37 kDa proteins disclosed by Fikrig are different proteins than the FlaA protein. In addition to the ambiguity which existed in the early prior art regarding nomenclature, the P37 protein of Fikrig has now been distinguished technically from FlaA:

> Genomic expression library screening with immune serum from patients or mice has resulted in the identification of at least two previously described 37-kDa proteins that are reactive with immune sera, including FlaA, an outer sheath protein of the periplasmic flagella, and P37, a lipoprotein that is preferentially expressed in vivo. (Feng, at p. 4172)

Accordingly, withdrawal of the 35 U.S.C. 102(a) rejection of claims 14-17 and 19-30 of Exhibit A are in order and respectfully requested.

Also, for the reasons discussed above, amended claims 14, 20 and 28 presented in the attached supplemental amendment (Exhibit B) are not anticipated by the references and would also allowable in the alternative.

D. Group II

Group II consists of independent claim 20 and dependent claims 21-26 as provided in Exhibit A. The claims are separately patentable from claim14. Claims 21-26 [illegible] with claim 20. Claim 20 reads as follows:

> providing freshly transformed host cells, constructing a DNA expression

vector containing an expressible FlaA encoding DNA sequence; transforming a suitable host cell with said expression vector; plating out transformed host cells; preparing large scale primary cell cultures from transformed host cells taken from a fresh transformant colony; and inducing FlaA or P37 protein expression from said host cells in culture to obtain a recombinant FlaA or P37 protein.

1. Claims 20-26 are definite under 35 USC 112, second paragraph because one of skill in the art would understand what is claimed in light of the specification.

Claims 20-26 in Exhibit A stand rejected as being vague and indefinite under 35 U.S.C. §112, second paragraph as follows:

(a) claim 20 in the recitation of both "recombinant FlaA or P37" protein; and

(b) claims 22 and 23 in the recitation of "partial amino acid sequence."

As discussed above, recitation of FlaA or P37 is defines the same protein, i.e., the recombinant FlaA protein. The art has now corrected the confusion in nomenclature and has distinguished P37 protein as being different from the FlaA protein of the invention. (See, Feng, p. 4172). No ambiguity exists in the recitation of "FlaA or P37" as found in the claims of Exhibit A and the Section 112 rejection should be withdrawn.

Also as discussed above, determination of "partial amino acid sequences" recited in claims 22 and 23 are within the skill of the artisan performing routine experimentation. It is respectfully submitted that the specification discloses the metes and bounds of the recitation in question. Claims 22 and 23 are not vague and indefinite and the Section 112 rejection should be withdrawn.

Alternatively, Applicants respectfully request the consideration and entry of the

2. Claims 20-26 are not anticipated by either Ge I or Ge II because neither reference teaches each and every element of the claim under 35 USC §102

Claims 20-26 were generally rejected under 35 USC §102 as being anticipated by Ge I or Ge II. The Examiner alleges that Ge II discloses producing the recombinant FlaA protein. In particular, the Examiner points out the steps of cloning FlaA into expression vectors using E. coli ... and the fusion protein, FlaA protein and maltose binding protein or glutathione S-transferase. (See, Final Office Action, Paper no. 16, p. 4, second paragraph). The Examiner's conclusion is based on incomplete analysis. The recombinant FlaA of the present invention is produced by a different method than that disclosed in Ge II.

Neither Ge I nor Ge II teach a method to produce an FlaA or P37 diagnostic reagent. Applicants have successfully produced a diagnostic reagent from FlaA protein that is derived from a fresh transformant colony. Applicants produce recombinant FlaA protein from transformed cell cultures as follows:

> constructing a DNA expression vector, containing an expressible FlaA encoding DNA sequence, transforming a suitable host cell with the expression vector, preparing large-scale cell cultures from fresh transformants of the host cell with the expression vector and not overnight starter culture and inducing FlaA protein expression from the large-scale cultures. (Specification p. 6, lines 5-15 and claim 20).

Applicants' method for producing the diagnostic reagent differs from Ge I or Ge II.

The Examiner's reliance on the Ge I or II references is improper over claims 20-26 because each and every element of the claimed method is not found in the references.

CONCLUSION

For all the above reasons, the rejections to claims 14-17 and 19-30 should be reversed. As a result claims 14-17 and 19-30 should be allowed.

Alternatively amended claims 14-17, 20-26, 28 and 29 as presented in the attached supplemental amendment (Exhibit B) should be allowed for the reasons presented above.

Respectfully submitted,

Date: June 22, 2001

By: _____
Lisa M.W. Hillman, Ph.D.
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APPENDIX A

14. A diagnostic reagent for early detection of Lyme disease comprising recombinant FlaA or P37 protein.

15. The diagnostic reagent of claim 14, said protein having the partial amino acid sequence as shown in SEQ ID NO:2.

16. The diagnostic reagent as in claim 15 wherein the recombinant FlaA or P37 protein is a fusion protein.

17. The diagnostic reagent as in claim 16 wherein the FlaA or P37 protein comprises a fusion partner that is approximately a 38 kDaT7 gene 10 product.

19. The diagnostic reagent of claim 14, said protein having the amino acid sequence of amino acids 1-319 of SEQ ID NO:2.

20. A diagnostic reagent for early detection of Lyme disease produced using a method for producing recombinant FlaA or P37 protein comprising: providing freshly transformed host cells; constructing a DNA expression vector containing an expressible FlaA encoding DNA sequence; transforming a suitable host cell with said expression vector; plating out transformed host cells; preparing large scale primary cell cultures from transformed host cells taken from a fresh transformant colony; and inducing FlaA or P37 protein expression from said host cells in culture to obtain a recombinant FlaA or P37 protein.

21. A diagnostic reagent as in claim 20 comprising the entire amino acid sequence encoded by the nucleic acid sequence as shown in SEQ ID NO:1.

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22. A diagnostic reagent as in claim 20 comprising the partial amino acid sequence as shown in SEQ ID NO:2.

23. A diagnostic reagent as in claim 20 comprising the partial amino acid sequence encoded by the nucleic acid sequence as shown in SEQ ID NO:3.

24. A diagnostic reagent as in claim 20 wherein the recombinant FlaA or P37 protein is a fusion protein.

25. A diagnostic reagent as in claim 20 wherein the recombinant FlaA or P37 protein comprises a fusion partner that is approximately a 38 kDa T7 gene 10 product.

26. A recombinant FlaA protein as in claim 20 wherein said transformed host cell is an E. coli cell.

27. A diagnostic reagent as in claim 14 comprising an amino acid sequence or fragment thereof selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

28. A host cell containing the nucleic acid sequence of claim 15 or a complement thereof.

29. An expression vector comprising the nucleic acid sequence of claim 15 or a complement thereof.

30. A diagnostic reagent for detection of Lyme disease comprising an amino acid sequence as in claim 15 which is substantially antigenic to B. burgdorferi antibodies.

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of: **Gilmore**	)	
	)	Group Art Unit: 1645
Serial No.: 09/004,395	)	
	)	Examiner: N. Minnifield
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	)	Docket No. 97,429

For: **RECOMBINANT P37/FLAA AS A DIAGNOSTIC REAGENT**

SUPPLEMENTAL AMENDMENT

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Appellants respectfully request entry of this amendment. It is believed that no fee is due in connection with this filing. However, if a fee is due, please charge our deposit account number 13-2490.

IN THE CLAIMS:

14. (Twice Amended) A diagnostic reagent for early detection of Lyme disease comprising a recombinant FlaA protein.

15. (Twice Amended) The diagnostic reagent of claim 14, wherein said protein comprises an amino acid sequence as shown in SEQ ID NO.:2.

17. (Twice Amended) The diagnostic reagent as in claim 16 wherein said fusion protein is approximately a 38 kDaT7 gene 10 product.

19. (Canceled)

20. (Amended) A diagnostic reagent for early detection of Lyme disease produced by a method comprising: providing freshly transformed host cells; constructing a DNA expression vector containing an expressible FlaA encoding DNA sequence; transforming a suitable host cell with said expression vector; plating out said transformed host cells; preparing large scale primary cell cultures from transformed host cells taken from a fresh transformant colony; and inducing FlaA protein expression from said host cells in culture to [obtain] produce a recombinant FlaA protein.

21. (Amended) A diagnostic reagent as in claim 20 wherein said diagnostic reagent is encoded by a nucleic acid sequence as shown in SEQ ID NO:1.

22. (Amended) A diagnostic reagent as in claim 20 comprising an amino acid sequence as shown in SEQ ID NO:2.

23. (Amended) The recombinant FlaA protein of claim 20 comprising an amino acid sequence encoded by the nucleic acid sequence as shown in SEQ ID NO:3.

24. (Amended) A diagnostic reagent as in claim 20 wherein said recombinant FlaA protein is a fusion protein.

25. (Amended) A diagnostic reagent as in claim 24 wherein said fusion protein is a 38 kDa T7 gene 10 product.

27. (Canceled)

28. (Amended) A host cell containing the nucleic acid sequence of claim 21 or a complement thereof.

29. (Amended) An expression vector comprising the nucleic acid sequence of claim 21 or a complement thereof.

30. (Canceled)

Remarks

The amendments cancel claims 19, 27, and 30, and place the remaining claims in better form for appeal. The amendments are necessary to respond to the Examiner's 35 U.S.C. §112, second paragraph rejections, which were made for the first time in the Final Office Action. The amendments were not earlier presented because the Appellants believed that the claims were in form for allowance. Appellants respectfully request entry of the Supplemental Amendment. A marked-up copy of the claims is attached.

Respectfully submitted,

Date: June 22, 2001

By: 

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Version with Markings to Show Changes Made

14. (Twice Amended) A diagnostic reagent for early detection of Lyme disease comprising a recombinant FlaA [or P37] protein.

15. (Twice Amended) The diagnostic reagent of claim 14, wherein said protein comprises an [having the partial] amino acid sequence as shown in SEQ ID NO.:2.

16. (Twice Amended) The diagnostic reagent as in claim 14 [15] wherein said [the] recombinant FlaA [or P37] protein comprises [is] a fusion protein.

17. (Twice Amended) The diagnostic reagent as in claim 16 wherein [the FlaA or P37 protein comprises a] said fusion protein [partner that] is approximately a 38 kDaT7 gene 10 product.

19. (Canceled)

21. (Amended) A diagnostic reagent for early detection of Lyme disease produced by [using] a method [for producing recombinant FlaA protein] comprising: providing freshly transformed host cells; constructing a DNA expression vector containing an expressible FlaA encoding DNA sequence; transforming a suitable host cell with said expression vector; plating out said transformed host cells; preparing large scale primary cell cultures from transformed host cells taken from a fresh transformant colony; and inducing FlaA [or P37] protein expression from said host cells in culture to [obtain] produce a recombinant FlaA [or P37] protein.

21. (Amended) A diagnostic reagent as in claim 20 wherein said diagnostic reagent is [comprising the entire amino acid sequence] encoded by a [the] nucleic acid sequence as

22. (Amended) A diagnostic reagent as in claim 20 comprising an [the partial] amino acid sequence as shown in SEQ ID NO:2.

23. (Amended) [A diagnostic reagent as in] The recombinant FlaA protein of claim 20 comprising [the partial] an amino acid sequence encoded by the nucleic acid sequence as shown in SEQ ID NO:3.

24. (Amended) A diagnostic reagent as in claim 20 wherein [the] said recombinant FlaA [or P37] protein is a fusion protein.

25. (Amended) A diagnostic reagent as in claim 24 [20] wherein [the] said [recombinant FlaA or P37 protein comprises a] fusion protein [partner that is approximately] is a 38 kDa T7 gene 10 product.

26. (Amended) A [recombinant FlaA protein] diagnostic reagent as in claim 20 wherein said transformed host cell is an E. coli cell.

27. (Canceled)

28. (Amended) A host cell containing the nucleic acid sequence of claim 21 [15] or a complement thereof.

29. (Amended) An expression vector comprising the nucleic acid sequence of claim 21 [15] or a complement thereof.

30. (Canceled) A diagnostic reagent for detection of Lyme disease comprising an amino acid sequence as in claim 15 which is substantially antigenic to B. burgdorferi antibodies.

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Lyme Arthritis Resolution with Antiserum to a 37-Kilodalton *Borrelia burgdorferi* Protein

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A 37-kDa protein from *Borrelia burgdorferi* (the agent of Lyme disease) was identified as a target for immune-mediated resolution of Lyme arthritis. Studies in a mouse model have shown that arthritis resolution can be mediated by antibodies (against unknown target antigens) within immune sera from actively infected mice. Immune sera from infected mice were therefore used to screen a *B. burgdorferi* genomic expression library. A gene was identified whose native product is a putative lipoprotein of approximately 37 kDa, referred to here as arthritis-related protein (Arp). Active and passive immunization of mice with recombinant Arp or Arp antiserum, respectively, did not protect mice from challenge inoculation. However, when Arp antiserum was administered to severe combined immunodeficient (SCID) mice with established infections and with ongoing arthritis and carditis, treatment selectively induced arthritis resolution without affecting the status of carditis or influencing the status of infection, including spirochetemia. The selective arthritis-resolving effect of Arp antiserum mimics the activity of immune serum from immunocompetent mice when such serum is transferred into SCID mice with established infections. The *arp* gene could not be amplified from unrelated *B. burgdorferi* isolates but hybridized with these isolates only under very-low-stringency conditions. Arp antiserum reacted against proteins of similar size in a wide range of *B. burgdorferi* isolates.

Lyme disease in humans, caused by tick-borne *Borrelia burgdorferi* infection, often presents as arthritis, which undergoes spontaneous resolution with periodic bouts of exacerbation over the course of months or years of persistent infection (32). A mouse model for Lyme disease follows a similar course (6) and has been utilized to show that arthritis resolution is an antibody-mediated event. When sera from actively infected immunocompetent mice that have undergone arthritis resolution (immune sera) are transferred to severe combined immunodeficient (SCID) mice with established infections and with arthritis and carditis, their arthritis resolves, but their carditis remains. Furthermore, immune serum treatment of infected SCID mice does not affect the status of their infection, and the mice remain spirochetemic (7, 8). Although antibody-mediated resolution of arthritis in human Lyme disease patients has not been proven, passively transferred sera from Lyme disease patients have been shown to protect recipient mice against challenge inoculation (23). This observation underscores the importance of humoral immune responses in both human Lyme disease and the mouse model.

Identification of the *B. burgdorferi* antigens that are targeted by arthritis-resolving antibodies in persistently infected hosts would greatly facilitate an understanding of Lyme disease pathogenesis. We therefore screened a *B. burgdorferi* strain N40 DNA genomic expression library with sera from actively infected mice and describe here 1 of 46 immunoreactive clones that induces arthritis-resolving antibody responses. Several *B. burgdorferi* antigens have been shown to induce partial or complete protective immunity against *B. burgdorferi* challenge, but this is the first report of a specific antigen that selectively modifies the course of Lyme arthritis during persistent infection.

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MATERIALS AND METHODS

Mice. Specific-pathogen-free, 3- to 5-week-old C3H/HeJ (C3H) and C3H/HeSmn-*scid* (C3H-*scid*) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

***B. burgdorferi*.** All mouse experiments used a low-passage clonal population of the N40 strain of *B. burgdorferi* (8). For each experiment, a frozen aliquot of *B. burgdorferi* was expanded at 33°C in BSK II broth (2). Spirochetes were grown to mid-log phase, assessed for viability, and then counted by dark-field microscopy using a bacterial counting chamber. Inocula were diluted to obtain the appropriate dose of spirochetes (depending upon the experiment, detailed below) in 0.1 ml of BSK II broth and then inoculated intradermally above the shoulders. The infection status of mice in all experiments was determined by culture of tissues (blood, spleen, urinary bladder, and tibiotarsus [ear]) in BSK II medium, as described earlier (8). For genetic and antigenic comparison among *B. burgdorferi* isolates, selected representatives of *B. burgdorferi* sensu lato were utilized, including *B. burgdorferi* sensu stricto strains N40 and 297 (closely related north-eastern U.S. isolates), *Borrelia bissettii* 25015 (genetically distinct from 119 species from the same geographic region as N40 and 297), *Borrelia afzelii* PKo (from Europe), and *Borrelia garinii* PBi (from Europe). Each of these strains represent clonal populations derived by repeated (three times) terminal dilution. The genetic identity of these clonal strains has been previously verified (6).

Immune sera and hyperimmune sera. Immune sera for screening the genomic expression library were obtained from C3H mice that were infected for 90 days following intradermal inoculation with 10^4 *B. burgdorferi* N40 cells. This infection dose has been shown to not induce a detectable antibody response unless the mouse is actively infected, a consideration of importance because active infection induces a different reactivity profile to *B. burgdorferi* than immunization (inoculated with high-dose inocula) with the organism (8). To assess serum antibody responses of infected mice against candidate recombinant proteins, groups of five C3H mice were inoculated intradermally with 10^4 *B. burgdorferi* N40 cells. Sera were collected from mice at 7, 14, 28, 60, and 90 days after inoculation. Infection of all mice was verified by culture of blood, spleen, urinary bladder, and inoculation site at the 90-day interval. Hyperimmune antisera were generated by subcutaneous immunization of C3H mice with 20 μg of recombinant protein in complete Freund's adjuvant (0.2-ml total volume) and boosted twice at 2-week intervals with 10 μg of protein in incomplete Freund's adjuvant.

Protective immunity. For challenge immunity experiments, C3H mice were actively immunized as described above. Prior to challenge of mice, serum antibody reactivity to Arp in the immunized group was verified by immunoblotting at serum dilutions of 1:1,000. Immunized mice were challenged intradermally with 10^4 *B. burgdorferi* cells. At 2 weeks after challenge, mice were assessed for infection by culture.

Arthritis-resolving immunity. Arthritis-resolving activity in hyperimmune antiserum was assessed in C3H-*scid* mice with established infection. C3H-*scid* mice were inoculated with 10^4 *B. burgdorferi* N40 cells, a high dose that assures infection of all mice and induces consistently severe arthritis. Depending upon

the experimental design (see Results), mice were boosted subcutaneously with 0.5 ml of emulsified recombinant protein-adjuvant mixture at similar intervals after infection and then assessed for infection status by culture and disease status by histology.

Genomic expression library, cloning, and expression. A λ ZAP II *B. burgdorferi* N40 genomic expression library was provided by R. A. Flavell, Yale University School of Medicine. The λ ZAP II phage contains pBluescript that can be excised and cloned directly with R408 helper phage (Stratagene, La Jolla, Calif.). Phages were incubated with *Escherichia coli*, protein expression was induced with 10 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and proteins were transferred to nitrocellulose membranes and then incubated with a 1:1,000 dilution of mouse immune serum. After washing, membranes were incubated with a 1:5,000 dilution of alkaline phosphatase-conjugated goat anti-mouse immunoglobulin antibodies (Sigma, St. Louis, Mo.), and bound antibodies were detected by color development with nitroblue tetrazolium (Stratagene) and 5-bromo-4-chloro-3-indolylphosphate (BCIP; Stratagene). Excision of the pBluescript plasmid from reactive clones was achieved using the R408 helper phage. DNA sequencing was performed at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale School of Medicine, and both strands were sequenced to ensure accuracy. The DNA sequence was analyzed using the MacVector program (Kodak, New Haven, Conn.).

Among 48 immunoreactive clones, the subject gene (and its product) was selected for evaluation, not only because it was immunoreactive but also because its sequence contained a Leu-X-Y-Cys consensus sequence at amino acids 9 to 12, presumably recognized by signal peptidase II, and a hydrophobic leader sequence. It was therefore likely to be expressed on the surface of *B. burgdorferi*. Furthermore, its predicted molecular mass of 37 kDa matched a size range on *B. burgdorferi* lysate immunoblots known to react with early-phase sera from infected mice which contain passive arthritis-resolving activity (5, 8, 10).

Sequence data were submitted to the GenBank Nucleotide Sequence Database (accession number AF050213) and matched the published DNA sequence of a B31 open reading frame (BBF01), with no assigned function, located on linear plasmid (lp) 28-1. There was 100% amino acid identity, with three nucleotide differences at bp 74 (T for N40 in lieu of a C for B31), 282, and 643 (A for N40 in lieu of G for B31) (24). A partial sequence (180 bp shorter at the C terminus due to a premature stop codon resulting from a single extra nucleotide insertion) was discovered by genomic library screening with mouse sera and was published previously (33). Because we can now ascribe function to the full-length gene product, we suggest here the name of arthritis-related protein (Arp).

The *arp* gene, lacking the sequence encoding for the hydrophobic N-terminal leader region (amino acids 1 to 12), was amplified by PCR from template DNA of the immunoreactive clone by using oligonucleotide primers based on their DNA sequences. The primers corresponded to nucleotides 37 to 73 and 151 to 873 of the gene. Elimination of the signal sequence increased the likelihood that the recombinant protein would be soluble when expressed, as previously described for the purification of OspA (14). The amplified *arp* gene was cloned in frame with glutathione *S*-transferase (GST) into pMX, a pGEX-2T vector (Pharmacia, Piscataway, N.J.) with a modified polylinker (20). The PCR-amplified DNA sequence of *arp* was confirmed by comparison with the original insert.

Another gene (*p37*) and its product were also identified and cloned by screening the genomic expression library (GenBank accession number AF032553). The *p37* gene is located on linear plasmid 56 (24), and its amino acid sequence suggests that it, like Arp, may be a lipoprotein. P37 protein, devoid of its leader sequence, was generated in an identical fashion as for Arp.

Recombinant protein. *E. coli* DH5α cells containing recombinant plasmids were grown to an optical density at 600 nm (OD_{600}) of 0.5 (ca. 3 h), and the recombinant GST fusion proteins were induced with IPTG at a final concentration of 1 mM (3 h). Bacterial cells were centrifuged at 4,000 rpm for 20 min, and pellets were washed with phosphate-buffered saline (PBS) and then dissolved in a 1:10 volume of PBS with 1% Triton X-100. The mixtures were sonicated and centrifuged at 10,000 rpm. Coomassie blue-stained polyacrylamide gels showed that GST-recombinant fusion proteins were soluble. The supernatants containing GST-recombinant fusion proteins were loaded onto glutathione-Sepharose 4B columns (Pharmacia), and then 25 U of thrombin was added to the columns and incubated at room temperature for 3 h to remove the GST partner. The Arp and P37 recombinant proteins were eluted and collected from columns, free of their GST fusion partner, and dialyzed against PBS three times (31).

Enzyme-linked immunosorbent assay (ELISA) and immunoblotting. A total of 100 μl of 1 μg of recombinant Arp or GST in carbonate coating buffer (pH 9.6) per ml was placed in 96-well plates (Becton Dickinson Labware, Franklin Lakes, N.J.) overnight at 4°C and then washed with PBS with 0.1 M Tween 20 (PBST) four times. Then, 150 μl of 1% bovine serum albumin in PBS was added to each well and incubated for [illegible] room temperature. [illegible] [illegible] including uninfected normal mouse serum [illegible] control, were serially diluted three times from an initial dilution of 1:100 [illegible] PBST added to the plates at 4°C overnight and then washed four times with PBST. Anti-mouse immunoglobulin G (IgG) linked with peroxidase (Sigma) at 1:5,000 was added to the plates at room temperature for another 2 h. Plates were washed with PBST four times, and 1 mg of p-nitrophenyl phosphate per ml was added to the plates. Plates were incubated at room temperature for 30 min and read on a Kinetic Microplate Reader (Molecular Devices, Sunnyvale, Calif.) at OD_{405}. Values subtracted from background reactivity against normal mouse serum. For immunob-

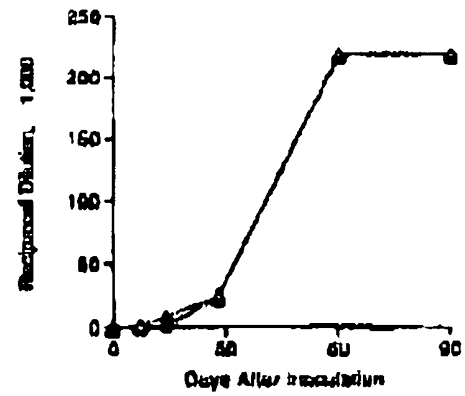

FIG. 1. Anti-Arp (open circles) compared to anti-*B. burgdorferi* (triangles) IgG ELISA titers (expressed in reciprocal serum dilutions) in sera from C3H mice at intervals after intradermal inoculation with *B. burgdorferi*. Nearly identical curves were obtained with the same sera tested against both antigens, indicating that active infection stimulated a strong immune response to native Arp that can be measured with the recombinant protein as antigen.

lotting, Arp, GST, or *B. burgdorferi* lysates were resolved in sodium dodecyl sulfate (SDS)–12% polyacrylamide gels by electrophoresis and then transferred to nitrocellulose membranes, which were cut into strips. The strips were probed with test mouse sera. The secondary antibody was alkaline phosphatase-conjugated goat anti-mouse IgG (Stratagene).

Histology. Rear legs and hearts were fixed in neutral buffered formalin (pH 7.2), processed by routine histologic techniques, stained with hematoxylin and eosin, and blindly examined without knowledge of experimental design or treatment group. Arthritis prevalence was assessed by microscopic examination of the tibiotarsal joints from both rear legs of each mouse, and carditis prevalence was assessed by microscopic examination of the heart base (aorta and surrounding tissue) for evidence of inflammation. Arthritis severity was scored on a scale of 0 (negative) to 3 (severe), as described earlier (7, 8).

Genomic DNA purification. Spirochetes were grown to 10^8 spirochetes/ml in a total volume of 100 ml and then harvested by centrifugation at 3,500 rpm (900 × *g*) for 20 min. Pellets were washed with 1 ml of ice-cold PBS and centrifuged at 10,000 rpm for 2 min. Supernatants were discarded, and pellets were dissolved in 1 ml of digestion buffer (100 mM NaCl, 10 mM Tris [pH 8], 25 mM EDTA, 0.5% SDS, 0.1 ml of proteinase K per ml) at 50°C overnight. Then, 1 ml of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the mixture and vortexed for 20 s. The mixtures were centrifuged at 14,000 rpm for 5 min, and the aqueous phase was collected. A total of 100 μl of 3.5 M sodium acetate and 2 ml of 100% ethanol was added to the aqueous phase. The mixtures were frozen at −70°C for at least 3 h, and then DNA was ethanol precipitated, air dried, and dissolved in double-distilled water.

Southern blotting. Genomic DNA was purified from *B. burgdorferi* strains N40, B31, 297, PKo, and PBi. A total of 16 μg of DNA from each strain was digested with *Eco*RI and run on 1% agarose gels. DNA was transferred to Hybond-N⁺ nylon filters (Amersham Pharmacia, Piscataway, N.J.) and hybridized with full-length *arp* DNA as the probe using ECL System (Amersham Pharmacia) as recommended by the manufacturer. Hybridization was performed at either low stringency (37°C) overnight, followed by a primary wash at 42°C, or moderate stringency (42°C overnight, followed by a primary wash at 55°C).

RESULTS

Serum antibody reactivity to Arp. Immune sera from actively infected mice were verified to contain IgG antibody reactivity to recombinant Arp antigen by ELISA as early as 7 days after infection, with rising titers through 90 days of active infection (Fig. 1). Thus, native Arp was immunologically recognized during early infection and elicited a strong antibody response, confirming that Arp is a major immunogen during the early phase of infection with *B. burgdorferi*.

Assessment of protective immunity induced by Arp immunization. Because immune sera from persistently infected mice have been shown to contain protective antibodies (5, 6), we sought to determine whether immunization with recombinant Arp would protect mice against challenge with of *B. burgdorferi*. The results indicated that mice could not be protected by either active or passive immunization. Groups of five mice

TABLE 1. Arthritis resolution in *B. burgdorferi*-infected SCID mice treated with Arp-hyperimmune antiserum compared to infected SCID mice treated with P37 (a non-arthritis-resolving antigen)- or GST (control)-antiserum[a]

Antiserum group	Culture (no. positive/ total no.)		Tibiotarsal arthritis (mean no. [±SD])		Carditis prevalence (no. positive/ total no.)
	Blood	Bladder	Prevalence	Severity	
GST (control)	4/4	3/3	2.0 ± 0	1.5 ± 0.6	4/4
P37 (control)	4/4	4/4	2.0 ± 0	1.3 ± 0.5	4/4
Arp	4/4	3/3	0.5 ± 0.6[b]	0.3 ± 0.5[c]	4/4

[a] Groups of four C3H-*scid* mice were infected with *B. burgdorferi* N40 for 6 days and then treated with 0.3 ml of Arp-, P37-, or GST-antisera on days 6 and 10. The infection status (culture) is indicated. The tibiotarsal arthritis prevalence (among both tibiotarsi) and arthritis severity (mean score of both tibiotarsi) are summarized as mean ± SDs. The carditis prevalence is also indicated. Dissemination less than 4 are due to bacterial contamination of cultures.

[b] $P < 0.01$ (chi-square test).

[c] $P < 0.01$ (unpaired Student's *t* test).

were actively immunized with recombinant Arp or GST (controls), antibody titers were verified, and then mice were challenged with *B. burgdorferi* N40. At 2 weeks after challenge, all mice in both treatment groups were infected, demonstrating no protective effect. A confirmatory experiment, in which groups of five C3H mice were passively immunized with 0.1 ml of Arp- or GST-hyperimmune sera and then challenged with *B. burgdorferi*, also revealed no evidence of protection.

Assessment of arthritis-resolving activity in Arp-antiserum. Because sera from persistently infected mice have been shown to contain arthritis-resolving antibodies (7, 8), we next sought to determine if Arp-antiserum would induce arthritis resolution in infected C3H-*scid* mice with progressive arthritis. Groups of four C3H-*scid* mice were inoculated with *B. burgdorferi* N40. At 6 and 10 days after inoculation, mice were treated subcutaneously with 0.3 ml of either Arp- or GST-hyperimmune antisera. A third group of mice was passively immunized with hyperimmune antiserum against an irrelevant but similar-molecular-weight *B. burgdorferi* protein (P37), which we have found to have no protective or arthritis-resolving activity. Mice were assessed for infection by culture and for disease by histology at 14 days after inoculation.

Arp-antiserum significantly reduced both tibiotarsal arthritis prevalence and severity compared with P37- and GST-hyperimmune antisera (Table 1). All mice in all three groups were culture positive, including blood (spirochetemia). Remarkably, although there was a significant reduction in both the prevalence and severity of arthritis compared to controls, antiserum treatment had no effect upon carditis. The experiment was repeated, using groups of four C3H-*scid* mice treated with Arp- or GST-hyperimmune antisera. There was arthritis amelioration in Arp-antiserum-treated mice (mean prevalence ± standard deviation (SD), 1.3 ± 0.5; mean severity ± SD, 0.8 ± 0.5) compared to GST-antiserum-treated controls (mean prevalence, 2.0; mean severity ± SD, 1.5 ± 0). As before, all mice remained culture positive and spirochetemic, and all mice had active carditis.

To further confirm the arthritis-resolving effects of Arp-antiserum, we next infected C3H-*scid* mice, as above, and then administered Arp- or GST-antiserum treatment on days 12, 18, and 24. This experiment differed from the previous experiments in that the C3H-*scid* mice were allowed to be infected longer (12 versus 6 days), thereby allowing more severe arthritis to develop, and then treating the mice with three doses (rather than two) of antisera and examining them for arthritis at a later interval (28 versus 14 days). As expected, mice

TABLE 2. Arthritis resolution in *B. burgdorferi*-infected SCID mice treated with Arp-hyperimmune antiserum compared to infected SCID mice treated with GST-antiserum[a]

Antiserum group	Culture (no. positive/ total no.)		Arthritis (mean no. [±SD])		Carditis prevalence (no. positive/ total no.)
	Blood	Bladder	Prevalence	Severity	
GST (control)	5/5	5/5	2.0 ± 0	2.0 ± 0.3	5/5
Arp	5/5	5/5	1.8 ± 0.5	1.1 ± 0.5[b]	5/5

[a] C3H-*scid* mice were infected with *B. burgdorferi* N40 for 12 days, an interval in which arthritis and carditis become well established, and then treated with 0.3 ml of Arp- or GST-antiserum on days 12, 18, and 24. Infection status (culture) is indicated. The arthritis prevalence (among both tibiotarsi) and arthritis severity (mean of both tibiotarsi) are summarized as means ± SDs. The carditis prevalence is also indicated.

[b] $P < 0.01$ (unpaired Student's *t* test).

treated with Arp-antiserum had less-severe arthritis compared to GST-antiserum-treated control mice (Table 2). As before, infection status, including spirochetemia, and carditis were not affected by treatment. Arthritis prevalence was not affected, since residual inflammation remained (and was scored positive, albeit less severe) in these mice with more advanced disease.

Arp among *B. burgdorferi* sensu lato strains. Because *B. burgdorferi* belongs to a large genospecies complex, we next sought to determine if Arp was conserved or unique among a broad array of *B. burgdorferi* sensu lato species, including strains N40, B31, 25015, PKo, and PBi. We first attempted to amplify the *arp* gene from target DNA of each *B. burgdorferi* strain by using the primers corresponding to nucleotides 17 to 73 and 951 to 975 described above. A product was amplified from N40 and B31 but not from the other strains. We next performed Southern blottings, in which genomic DNA was transferred to nylon filters and then blotted with N40 *arp* DNA as a probe, using relatively moderately stringent conditions (42°C overnight, followed by a primary wash at 55°C). Single bands of different sizes were detected from strains N40 and B31 but not from strains 25015, PKo, or PBi (Fig. 2). We next attempted to hybridize *arp* DNA with target DNA from these strains, using very-low-stringency conditions (37°C overnight, followed by a primary wash at 42°C). Under these very-low-stringency conditions, *arp* DNA hybridized with all strains. These results suggested that strains N40 and B31 possess single copies of *arp* genes in keeping with published B31 genomic

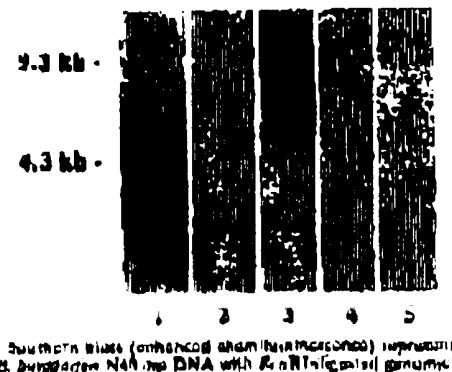

FIG. 2. Southern blots (enhanced chemiluminescence) representing hybridization of *B. burgdorferi* N40 *arp* DNA with *B. burgdorferi*-infected genomic DNA from *B. burgdorferi* N40 (lane 1), *B. afzelii* Zs7.1 (lane 2), *B. garinii* 20047 (lane 3), *B. garinii* PKo (lane 4), and *B. garinii* PBi (lane 5).

sequence data. The results also suggest that homologous genes among *B. burgdorferi* sensu lato strains are distinctly related.

To further evaluate Arp among *B. burgdorferi* sensu lato strains, we performed immunoblots on N40, B31, 25015, PKa, and PBi lysates that were transferred to nitrocellulose filters and probed with Arp antiserum. Reactivity against 37- to 38-kDa proteins was detected among all *B. burgdorferi* strains (Fig. 1). These results suggest that *arp* genes were different on the DNA level but that all strains shared at least some common antigenic epitopes of similarly sized proteins.

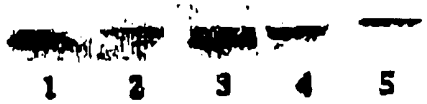

FIG. 1. Immunoblot (alkaline phosphatase) representing reactivity of *B. burgdorferi* anti-Arp antiserum against lysates of *B. burgdorferi* N40 (lane 1), *B. garinii* 25015 (lane 2), *B. burgdorferi* B31 (lane 3), *B. afzelii* PKa (lane 4), and *B. garinii* PBi. Antiserum recognized antigens of approximately the same molecular mass in all *B. burgdorferi* sensu lato strains.

DISCUSSION

We describe here a 37-kDa arthritis-related protein (Arp) that elicits a strong antibody response during early infection with *B. burgdorferi* and also is capable of generating arthritis-resolving antibody upon immunization of mice with the recombinant protein. It appears that humans (and mice) infected with *B. burgdorferi* develop antibody to one or more 37-kDa antigens on *B. burgdorferi* lysates, as determined by immunoblotting (1, 18, 25) during early infection. Genomic expression library screening with immune serum from patients or mice has resulted in the identification of at least two previously described 37-kDa proteins that are reactive with immune sera, including FlaA, an outer sheath protein of the periplasmic flagella (25), and P37, a lipoprotein that is preferentially expressed in vivo (21). We report here two additional immunoreactive 37-kDa lipoproteins, one of which we have designated Arp. These findings reinforce the need to name genes and gene products based upon function rather than molecular weight to avoid confusion.

The gene sequence of Arp matched the sequence of a B31 open reading frame located on lp28-1 (24). A partial sequence (150 bp shorter at the C terminus due to a premature stop codon resulting from a single extra nucleotide insertion) was discovered by genomic library screening with mouse sera and was published previously (33). The partial gene product was named ErpT, but the designation of either ErpT or Arp as belonging to the E- or F-related protein (Erp) paralogous family may be inappropriate. First, Arp (or ErpT) does not share the highly conserved upstream homology region characteristic of the Erp family. Second, the *arp* gene is located on lp28-1 and not on cp32/18, which is typical for the Erp family. The only similarity between Arp and members of the Erp family is within the leader sequence (2, 11, 33, 34). This suggests a remote evolutionary relatedness of Arp to Erps, but Arp clearly falls outside of the characteristics of the Erp family as most recently defined (2, 11). For these reasons and because we can now ascribe function to the full-length gene product, we suggest the name of arthritis-related protein (Arp).

It is notable that in a previous study on the truncated (ErpT) form of Arp, active immunization with the ErpT recombinant protein failed to induce protective or arthritis-resolving immunity in mice (33). Comparison of those findings with the current study is valid, since one of the authors (S. W. B.) performed the arthritis evaluation in both studies. However, in the previous study on ErpT, mice were hyperimmunized with the truncated recombinant protein and found to be fully susceptible to challenge infection and developed arthritis to the same degree as control mice. Although the earlier study did not assess arthritis by passive immunization, active immunization would have abrogated the development of arthritis in the mice. It remains to be determined if the arthritis-resolving epitopes of Arp are indeed located in the carboxy terminus of the protein.

Analysis of *erpT* mRNA in selected tissues of infected mice suggested that *erpT* (and therefore Arp) was expressed by spirochetes in the joints, heart, and spleen but not by spirochetes in skin (33). However, in the present study, the disease-resolving activity of Arp antiserum was selective for joints, without an effect on heart disease. This may seem in conflict with the observation that ErpT (Arp) is also expressed in the heart, but it is important to note that whether or not the antigenic targets are the same for immune-mediated carditis resolution, carditis resolution is not effectively mediated by antibody compared with arthritis (7, 8). Clearly, quantitative kinetic studies are needed to examine these issues and are under way.

It may seem incongruous that antiserum to a single *B. burgdorferi* protein (Arp) can selectively induce arthritis resolution without invoking protective immunity, altering infection status (including spirochetemia) or influencing the states of carditis, but this, in fact, is the expected result and validates our findings with immune sera from infected mice. When immune sera from actively infected mice (containing undefined antibody) are passively transferred to naive mice, very small quantities of such sera will protect the mice against high-dose challenge (5, 8). We believe that the protective activity in immune sera is likely to be due to antibody against decorin-binding protein A (DbpA) (30, 36, 37). Active and passive immunization with DbpA elicits protective immunity but does not alter infection or affect arthritis or carditis in actively infected mice (20). When immune sera are transferred to *CH-scid* mice with established infections and with existing joint and heart disease, immune sera induce arthritis resolution, but mice continue to be spirochetemic, and their carditis remains unaffected by serum treatment (7, 8). Our current data, which identify Arp as the target for selective arthritis-resolving antibody, and other studies, which identify DbpA as a target for protective antibody, lend credence to the hypothesis that protective immunity, arthritis-resolving immunity, and carditis-resolving immunity, which all evolve in actively infected immunocompetent mice, are separate phenomena that may involve different *B. burgdorferi* target antigens or immune responses. Indirect evidence is also available suggesting that arthritis-resolving activity in sera from mice infected with different *B. burgdorferi* sensu lato strains may be strain specific (4), thus confirming our current findings of Arp antigenic cross-reactivity among strains but distant relatedness of the genes. It remains to be determined if Arp is the only antigen responsible for the arthritis-resolving activity in the immune serum of actively infected mice.

It is now certain that *B. burgdorferi* is a very dynamic organism which up- and downregulates different genes in different environments. For example, OspA is abundantly expressed by *B. burgdorferi* within the midgut of flat (resting) ticks but is repressed upon onset of feeding and entry into the mammalian host, whereas Arp is upregulated during tick feeding and in vivo (15–17, 30). Other proteins are selectively expressed in the mammalian host, including the Erp paralogue family, fibronectin-binding protein, DbpA/B, and Arp (based upon ErpT findings). Some of these gene products appear to be upregulated at different times during infection or within the context of different tissues (13–14, 21, 25, 29, 33–35, 37, 38).

Arp is the first *B. burgdorferi* gene product to be identified that elicits a selective Lyme disease-resolving immune response during persistent infection of the host, thereby mimicking the biological behavior of immune sera from infected mice. A notable exception is a report that described the treatment of SCID mice, infected with *B. burgdorferi* ZS7 (a European isolate), with antiserum to outer surface protein C (OspC). These mice were cured of infection by such treatment (39), suggesting that ZS7 may constitutively express OspC during infection, thereby making it uniquely vulnerable to OspC antibody. OspC active and passive immunization of mice challenged or infected with *B. burgdorferi* N40 had neither protective, arthritis-resolving, nor curative effects (8, 11). The selective arthritis-resolving effects that we have demonstrated with Arp-antiserum precisely fit the effect of passively transferred immune serum, thereby validating the biological significance of Arp in Lyme disease.

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of: **Gilmore**	)	
	)	Group Art Unit: 1645
Serial No.: 09/004,395	)	
	)	Examiner: N. Minnifield
Filed: December 23, 1996	)	
	)	Docket No. 97,429

For: **RECOMBINANT P37/FLAA AS A DIAGNOSTIC REAGENT**

BRIEF ON APPEAL

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